

Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for Testing Growth-Promoting
Qualities of Fluid Thioglycollate Medium with Beef
Extract Using *Clostridium chauvoei* Spores as the
Indicator Organism

Date: March 3, 1999
Supersedes: March 9, 1978
Number: STSAM0901.01
Standard Requirement: 9 CFR, Part 113.25
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Supplemental Assay Method for Testing Growth-Promoting Qualities of Fluid
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1. Introduction

This is a Supplemental Assay Method (SAM) for testing Fluid Thioglycollate Medium with Beef Extract for growth-promoting qualities as required in the Code of Federal Regulations, Title 9 (9 CFR), Part 113.25(b).

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

- 2.1.1 30°-35°C incubator
- 2.1.2 Sterile disposable cotton-plugged pipettes
- 2.1.3 Sterile 10-ml disposable syringes, with needles
- 2.1.4 Biosafety cabinet
- 2.1.5 Revco freezer
- 2.1.6 Magnetic stirrer

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

- 2.2.1 Indicator organism: Use *Clostridium chauvoei* spores (prepared according to IRP 206 by the Biologics Bacteriology (BB) section of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent organisms as specified in the current United States Pharmacopoeia (USP).

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2.2.2 Media: Fluid Thioglycollate Medium with 0.5% Beef Extract (FTM/BE), 40 ml in 25 x 200-mm tubes, and Soybean Casein Digest Medium (SCDM) or Trypticase Soy Broth (TSB). See **Section 9.1** for media formulations.

2.2.3 Diluent: Sterile 0.85% NaCl solution (**Section 9.1.3**).

3. Preparation for the test

3.1 Personnel qualifications/training

Personnel must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. Personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent; and training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn biosafety cabinets on at least 1 hr before preparing positive control reagents or testing media for growth promotion.

3.2.2 Monitor incubators daily for temperature according to the current version of GDOCSOP0001.

3.2.3 Monitor freezers and coolers used for the storage of reagents and controls for temperature daily according to the current version of GDOCSOP0003.

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3.3 Preparation of *Clostridium chauvoei* reagent

3.3.1 Request a vial of *C. chauvoei* IRP 206 from the BB section of the CVB-L.

3.3.2 Place 1 ml of this *C. chauvoei* culture into 100 ml of 0.85% saline (**Section 9.1.3**).

3.3.3 Mix this 101 ml on a magnetic stirrer.

3.3.4 Place 1.5-ml aliquots of this stock culture (spore suspension) into small sterile screw-cap tubes and then store frozen at -70°C.

4. Performance of the test

4.1 Establishing the dilutions to be used in testing new media

4.1.1 Remove a vial of the newly prepared *C. chauvoei* stock culture from the freezer and thaw rapidly.

4.1.2 Make tenfold dilutions of the stock culture by using a 1-ml pipette to place 1 ml of the stock culture in 9 ml of SCDM (10^{-1} dilution).

4.1.3 Mix by inverting the tube several times.

4.1.4 Using a 1-ml pipette, transfer 1 ml of 10^{-1} culture dilution to 9 ml of SCDM (10^{-2} dilution). Mix as before and continue the procedure until 10^{-10} dilution is prepared.

4.1.5 Incubate the *C. chauvoei* dilution tubes for 24 to 48 hr at 30°-35°C.

4.1.6 Examine the tubes visually for growth to establish the growth endpoint of the stock culture.

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4.1.7 After establishing the growth endpoint, use this dilution and the next lower dilution for testing the new media.

4.1.8 Next, data must be accumulated to confirm these dilutions (**Section 4.1.7**) as the proper ones for testing new media with this stock culture. To gather this data, test 10 batches of the media (FTM/BE) with 10 tubes per dilution or 5 batches of each media with 20 tubes per dilution. Use a separate vial of stock culture for each batch.

4.1.9 Growth is expected in 9 or 10 tubes inoculated with the lower dilution (**Section 4.1.7**) and in less than 10 tubes inoculated with the higher dilution (**Section 4.1.6**).

4.1.10 If all of the test batches (**Section 4.1.8**) give the expected number of tubes with growth (**Section 4.1.9**), then these dilutions will be used in testing new media. If not, higher or lower dilutions are confirmed for testing new media as outlined in **Sections 4.1.8 and 4.1.9**.

4.2 Testing the media

4.2.1 Test each batch of FTM/BE prepared for sterility testing for growth-promoting qualities with the *C. chauvoei* spores. Thaw a tube of *C. chauvoei* rapidly.

4.2.2 Transfer 1 ml of the *C. chauvoei* stock culture to a tube with 9.0 ml of SCDM (10^{-1} dilution).

4.2.3 Mix the 10^{-1} dilution by shaking.

4.2.4 Transfer 1.0 ml of the 10^{-1} culture dilution to 9.0 ml of SCDM (10^{-2} dilution) using a sterile 1-ml pipette. Mix as before and continue tenfold dilutions until the last 2 dilutions.

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4.2.5 Use the last 2 dilutions (established in **Sections 4.1.6 & 4.1.7**) to inoculate the media being tested for growth promotion.

4.2.6 Using a sterile 10-ml syringe with needle, deposit 1.0 ml of the last dilution into each of 10, 25 x 200-mm tubes containing 40 ml of FTM/BE. Refill the same syringe with the lower dilution of culture and deposit 1.0 ml into each of 10, 25 x 200-mm tubes containing 40 ml of FTM/BE.

4.2.7 Incubate all tubes (20) at 30°-35°C and observe for growth of the organism throughout the 14-day incubation period.

4.2.8 Thoroughly clean the work area after completing the entire procedure.

5. Interpretation of the test results

Growth is expected in 9 or 10 tubes inoculated with the lower culture dilution and in less than 10 tubes inoculated with the higher culture dilution. If the spore suspension has not deteriorated, and less than 9 or 10 tubes inoculated with the lower culture dilution contain growth, the growth-promoting quality of the medium is in question.

6. Report of test results

Record the results of these growth-promotion tests in the positive control log book next to the media control number for that batch of media.

7. References

7.1 Code of Federal Regulations, Title 9, Part 113.25, U.S. Government Printing Office, Washington, DC, 1998.

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8. Summary of Revisions

This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. The following is a list of significant changes made from the superseded protocol:

8.1 The dilution medium used for tenfold dilutions was changed from 0.85% Saline to SCDM.

8.2 Equivalent organisms (*Clostridium sporogenes* [American Type Culture Collection #11437]) specified in the USP are allowed.

9. Appendices

9.1 Media formulations

9.1.1 NVSL Media Formulation #10227

FLUID THIOGLYCOLLATE WITH BEEF EXTRACT

Fluid Thioglycollate Medium	29.5 g
QH ₂ O	1000 ml
Heat and add:	
0.5% Beef Extract (Difco)	5 g

Bring to a boil and dispense.
Autoclave 20 min at 121°C.

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9.1.2 NVSL Media Formulation #10423

TRYPTICASE SOY BROTH (TSB)
or
SOYBEAN CASEIN DIGEST MEDIUM (SCDM)

Trypticase Soy Broth 30 g
QH₂O 1000 ml
Autoclave 20 min at 121°C.
TSB and SCDM are 2 names for the same media formulation
from different media companies.

9.1.3 NVSL Media Formulation #30201

SALINE, 0.85% (NORMAL SALINE)

Sodium Chloride 8.5 g
QH₂O 1000 ml

Autoclave for 20 min at 121°C.